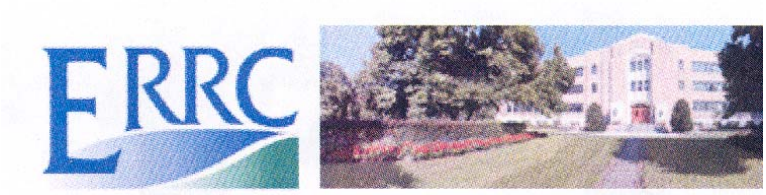

EASTERN REGIONAL RESEARCH CENTER
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
600 E. MERMAID LANE
WYNDMOOR, PA 19038
(215) 233-6400

Title: Inactivation of Escherichia coli O157:H7 on Inoculated Alfalfa Seeds with Ozonated Water and Heat Treatment

Author(s): R.R. Sharma, A. Demirci, L.R. Beuchat, and W.F. Fett

Citation: Journal of Food Protection (2002) 65(3): 447-451

Number: 7080

Inactivation of *Escherichia coli* O157:H7 on Inoculated Alfalfa Seeds with Ozonated Water and Heat Treatment

RATNA R. SHARMA,[1] ALI DEMIRCI,[1,2]* LARRY R. BEUCHAT,[3] AND WILLIAM F. FETT[4]

[1]*Department of Agricultural and Biological Engineering and* [2]*Life Sciences Consortium, The Pennsylvania State University, University Park, Pennsylvania 16802;* [3]*Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797; and* [4]*U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Plant Science and Technology Research Unit, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA*

MS 01-267: Received 27 July 2001/Accepted 14 October 2001

ABSTRACT

Alfalfa seeds inoculated with a five-strain mixture of *Escherichia coli* O157:H7 were immersed in water containing 4, 8, 10, and 21 ppm of ozone for 2, 4, 8, 16, 32, and 64 min at 4°C. Direct ozone sparging of seeds in water was used as an alternative mode of ozone treatment. Ozone-sparged seeds were also subsequently exposed to heat treatment at 40, 50, and 60°C for 3 h. Populations of *E. coli* O157:H7 on untreated and treated seeds were determined by spread plating diluted samples on tryptic soy agar supplemented with 50 μg/ml of nalidixic acid. Since *E. coli* O157:H7 was released from inoculated seeds during treatment with ozone, the rate of release of cells from inoculated seeds soaked in 0.1% peptone water for up to 64 min was also determined. The overall reduction of *E. coli* O157:H7 on seeds treated with ozonated water without continuous sparging ranged from 0.40 to 1.75 $\log_{10}$ CFU/g (59.6 to 98.2%), whereas reductions for control seeds were 0.32 to 1.03 $\log_{10}$ CFU/g (51.7 to 90.5%). Treatment with higher ozone concentrations enhanced inactivation, but contact time longer than 8 min did not result in significantly higher reductions ($P > 0.05$). For seeds treated by ozone sparging, a 1.12-$\log_{10}$ CFU/g (92.1%) reduction was achieved using a 2-min contact time, and a 2.21-$\log_{10}$ CFU/g (99.4%) reduction was achieved with a 64-min contact time. The corresponding reductions for control seeds were 0.71 $\log_{10}$ CFU/g (79.5%) and 2.21 $\log_{10}$ CFU/g (99.4%), respectively. Treatment of ozone-sparged seeds at 60°C for 3 h reduced the population to an undetectable level by direct plating (4 to 4.8 $\log_{10}$ CFU/g), although survivors were detected by enrichment. Ozone did not have a detrimental effect on seed germination percentage.

Seed sprouts, a part of traditional Oriental cuisine, have now gained popularity in many parts of the world, including Europe and the United States, because of their nutritive value. There has been an increase in consumer demand for mung bean, alfalfa, rice, wheat, and other seed sprouts that are prepared either commercially or at home *(14)*. Unfortunately, sprouts have recently been implicated in outbreaks of foodborne illness. Between 1995 and 1998, there were nine outbreaks of *Escherichia coli* O157:H7 and *Salmonella* infections associated with commercial sprouts *(11)*.

Sprouts are produced by soaking seeds in water, followed by intermittent irrigation during approximately 3 to 7 days at 20 to 25°C, with the growth period being dependent on the sprout type. They are commonly consumed raw or slightly cooked, e.g., in soups or mixed with other vegetables. These conditions increase potential health hazards associated with sprout consumption *(11, 15)*. Pathogens involved in multiple international outbreaks linked to raw sprouts include *E. coli* O157:H7, various serotypes of *Salmonella*, and *Bacillus cereus (7, 11, 18)*. Seeds are the likely source of contamination in most of the outbreaks, although contamination may also result from using contaminated water during sprout production or by mishandling during harvesting, packaging, or distribution or in the home.

Researchers have investigated chemical and heat treatments to reduce or eliminate pathogens inoculated onto seeds and sprouts. However, no single treatment has been found to completely eliminate pathogens under experimental conditions *(11)*. Hydrogen peroxide, sodium hypochlorite, calcium hypochlorite, ethanol, and organic acids have been tested for effectiveness in killing pathogens on seeds and sprouts *(2, 10)*. Taormina and Beuchat *(16, 17)*, in studies of the efficacy of various chemical treatments in killing *E. coli* O157:H7, reported that none of the chemical treatments evaluated satisfactorily reduced populations of *E. coli* O157:H7 on alfalfa seeds and sprouts. They concluded that recommended procedures for sanitizing alfalfa seeds fail to eliminate the pathogen. Treatment of alfalfa sprouts with antimicrobials was reported to be ineffective in eliminating *Salmonella* and *Vibrio cholerae* on alfalfa sprouts *(14)*. Although good manufacturing practices and hazard analysis and critical control point approaches can reduce the risk of contamination, there is still a need to develop highly effective methods to prevent sprout-associated infections.

Ozone has certain characteristics that make it attractive for use as a sanitizer in food processing. It is a strong antimicrobial agent with high reactivity and penetrability

* Author for correspondence. Tel: 814-863-1098; Fax: 814-863-1031; E-mail: demirci@psu.edu.

and spontaneously decomposes to a nontoxic product (oxygen). Ozone has been used with varied success to inactivate microorganisms on meat, poultry, eggs, fish, fruits, vegetables, and dry fruits *(9)*. Ozonation is preferred as a treatment to sanitize public water supplies in Europe. Application of ozone in the food industry in the United States has been limited, although its use has been approved by an expert panel as generally recognized as safe *(5, 13)*. On 26 June 2001, the Food and Drug Administration approved safe use of ozone in gaseous and aqueous phases as an antimicrobial agent for treatment, storage, and processing of foods *(3)*, which will increase ozone utilization in the food industry.

Ozonation involves onsite production of low concentrations of ozone gas from ambient air by means of an ozone generator. Ozone gas is injected immediately into a water or gas stream, where it dissolves and/or mixes to achieve its desired antimicrobial effect *(12)*. Ozone decays quickly in water, so its use as a sanitizer can be considered as a process rather than a food additive, with no safety concerns about consumption of residual ozone in treated foods. However, to effectively use ozone to decontaminate food products such as seed sprouts, it is necessary to properly define the mode of introduction of ozone to treatment water, optimum ozone concentration (ppm), contact time, and other treatment conditions. It is for this reason that the present study was undertaken to compare the effects of treatment of alfalfa seeds with ozonated water, followed by heat treatment, on inactivation of *E. coli* O157:H7 on alfalfa seeds. The effect of ozone on seed viability was also investigated to determine commercial applicability of ozone as a disinfectant in the sprout industry.

MATERIALS AND METHODS

Preparation of *E. coli* O157:H7 inoculum. Five strains of enterohemorrhagic *E. coli* O157:H7 resistant to nalidixic acid were obtained from the Center for Food Safety, University of Georgia. The strains were 932 (human isolate), 994 (salami isolate), E0018 (calf fecal isolate), H1730 (human isolate from outbreak associated with lettuce), and F4546 (human isolate from an outbreak associated with alfalfa sprouts). Cells were grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) supplemented with 50 μg/ml of nalidixic acid and 0.1% dextrose (TSBN) at 37°C for 18 h. The use of nalidixic acid minimized growth of microorganisms other than *E. coli* O157:H7 in enumeration media. A mixture of the five *E. coli* O157:H7 strains was prepared by combining 100 ml of each 18-h culture and centrifuging (Sorvall STH750, Kendro Lab Product, Newtown, Conn.) at 4°C and 3,300 × *g* for 15 min. The supernatant was decanted and the pellet was resuspended in 300 ml of sterile 0.1% peptone water before centrifuging again at 3,300 × *g* for 15 min at 4°C. The pellet was then resuspended in 1 liter of sterile 0.1% peptone water.

Contamination of alfalfa seeds. Alfalfa seeds (lot no. TY12) were obtained from International Specialty Supply (Cookeville, Tenn.). One kilogram of alfalfa seeds was soaked in the five-strain suspension of *E. coli* O157:H7 (~10^8 CFU/ml) for 1 min with gentle agitation. After the inoculum was decanted, seeds were placed on a sterile perforated tray lined with four layers of cheesecloth and dried in a laminar flow hood at room temperature (21 ± 1°C) for 24 h. Dried seeds with approximately 10^5 CFU/g of *E. coli* O157:H7 were sealed in plastic Ziploc bags and stored at 4°C until used within 1 week.

Preparation of ozonated water. Ozone gas (0.34 m^3/h) was generated using a lab-scale ozone generator (model no. H-50, Hess Machines International, Ephrata, Pa.) equipped with an oxygen concentrator. Two liters of sterile, deionized water at 4°C in a 2-liter Erlenmeyer flask fitted with a silicon stopper, inlet, and exit lines was sparged with ozone through a 10-μm stainless steel sparger for 1 h to attain 21 ppm of aqueous ozone. Excess ozone was passed through 2% potassium iodide solution to prevent ozone from being released into the environment. The sparging process was performed in a fume hood for safety purposes. Also, the temperature of water during ozonation was maintained at 4°C, since the solubility of ozone is higher at lower temperatures. The ozonated water so prepared was then diluted with sterile deionized water to obtain the desired concentration for treatments.

The concentration of ozone in the water was determined by direct measurement of UV absorption at 258 nm *(1, 4, 6)*. The formula used is given in equation 1.

$$c = A \times b/\epsilon \quad (1)$$

where c is the concentration of ozone in water (ppm, mg/liter); A is the absorbance value at UV 258 nm; b is the length of path of light, which is equal to the width of quartz cuvet (cm); and ϵ is the molar absorptivity, which is equal to 2,900 M^{-1} cm^{-1}.

Substituting values and conversion factors (48,000) balances the units to yield equation 2.

$$c = \frac{A \times 1 \times 48{,}000}{2{,}900} \text{ ppm} \quad (2)$$

Treatment of alfalfa seeds. The mode of introduction of ozone, ozone availability, and reaction time can influence the rate of cell inactivation. Two different methods for treating seeds with ozonated water, coupled with combinations of ozone concentration and exposure time, were evaluated for effectiveness in killing *E. coli* O157:H7.

Twenty-five grams of contaminated alfalfa seeds was soaked in 1 liter of ozonated water with continuous agitation using a motorized stirrer rod at a speed setting of 2 (model no. 4554-00, Cole Parmer Inst. Co., Barrington, Ill.). To investigate the effect of ozone concentration on lethality to *E. coli* O157:H7, water initially containing 4, 8, 10, and 21 ppm of ozone was used. These concentrations enabled a study of the effects of a range of ozone concentrations that could be produced with the lab-scale ozone generator. Alfalfa seeds were soaked in the ozonated water at each concentration for 2, 4, 8, 16, 32, and 64 min to determine the combination of concentration and reaction time that resulted in the highest reduction in number of *E. coli* O157:H7. Each experiment was replicated three times.

Continuous sparging of water in which alfalfa seeds inoculated with *E. coli* O157:H7 were immersed was also evaluated as a treatment to kill the pathogen. Ozone gas was directly sparged into 1 liter of sterile deionized water containing 25 g of inoculated seeds. Reduction in population of *E. coli* O157:H7 was determined for sparging times of 2, 4, 8, 16, 32, and 64 min. Seeds exposed to continuous sparging treatments were subsequently held in an incubator at 40, 50, or 60°C for 3 h to determine if a synergistic or additive effect occurred as a result of sequential treatments. Each experiment was replicated three times.

Microbiological analysis. To determine the initial population of *E. coli* O157:H7 on seeds before treatment with ozone, 10 g

of contaminated seeds was placed in 40 ml of sterile 0.1% peptone water in a stomacher 400 bag for 2, 4, 8, 16, 32, and 64 min. Seeds were pummeled for 30 s. The wash solution was serially diluted in sterile 0.1% peptone and surface plated (0.1 ml) in duplicate on tryptic soy agar supplemented with 50 μg/ml of nalidixic acid (TSAN; Difco). After incubating at 37°C for 24 h, presumptive *E. coli* O157:H7 colonies were enumerated.

Populations of *E. coli* O157:H7 on ozone-treated seeds were determined by placing the treated seeds (25 g) in 100 ml of sterile 0.1% peptone water followed by pummeling in a stomacher for 30 s, serially diluting in 0.1% peptone, and surface plating on TSAN. Colonies formed on plates inoculated with peptone wash samples from both untreated and treated seeds were randomly picked and subjected to *E. coli* O157 latex agglutination test (Oxoid) to confirm identity. Enrichment was performed by adding 100 ml of TSBN to the seed sample and incubating at 37°C for 48 h. The enriched cultures from samples in which no *E. coli* O157:H7 was detected were streaked on TSAN and incubated at 37°C for 24 h. Plates were examined for presumptive *E. coli* O157:H7 colonies, which were confirmed using O157 latex agglutination tests.

For each of the treatments in which ozone was not continuously sparged, sterile deionized water was included as a control treatment using the same temperature, time, and agitation conditions. The control for continuous sparging of ozone consisted of placing inoculated seeds in sterile deionized water at 4°C for the same exposure times as those used for seeds not subjected to continuous sparging.

Effect of ozone treatment on viability of alfalfa seeds. Alfalfa seeds treated with ozone and water were tested for viability, i.e., percentage capable of germination. Approximately 100 seeds were placed between two moistened filter paper discs in a petri dish. Water was applied by spraying from time to time during a 48-h period at 30°C to provide sufficient moisture for viable seeds to germinate. Seeds were visually examined after 48 h, and the percentage of germination was calculated.

Statistical analysis. All treatments were replicated three times. The results were analyzed using MINITAB (Minitab Inc., State College, Pa.) for analysis of variance and determining significant and nonsignificant differences in percent reduction in population of *E. coli* O157:H7 on alfalfa seeds subjected to each treatment. A 95% confidence level was used.

RESULTS AND DISCUSSION

Ozone treatment without continuous sparging. Treatment of inoculated alfalfa seeds with water containing initial concentrations of 4, 8, 10, and 21 ppm of ozone for up to 64 min resulted in reductions in population ranging from 0.40 to 1.75 $\log_{10}$ CFU/g (59.6 to 98.2%) (Table 1). The initial population was 4 to 5 $\log_{10}$ CFU/g. Longer contact time caused more *E. coli* O157:H7 to be released from the seeds and thus gave higher initial counts compared with shorter contact times. It was expected that moderate agitation during treatment would enhance release of *E. coli* O157:H7 from seeds and subsequent inactivation. However, this procedure reduced the stability of ozone and led to its rapid dissipation to oxygen, thereby reducing lethality to test cells.

The control treatment (0 ppm of ozone) resulted in a 0.32-$\log_{10}$ CFU/g (51.7%) reduction in population of *E. coli* O157:H7 within 2 min. Analysis of variance using a general linear model involving two-way interaction terms

TABLE 1. *Reduction in populations of* E. coli *O157:H7 on alfalfa seeds treated with water containing initial ozone concentrations of 0 to 21 ppm for up to 64 min*[a]

Contact time (min)	Population reduction ($\log_{10}$ CFU/g)				
	0 ppm	4 ppm	8 ppm	10 ppm	21 ppm
2	B 0.32 B	B 0.40 B	B 0.62 A	A 0.90 A	A 0.78 A
4	B 0.32 B	B 0.51 A	B 0.61 A	A 0.82 A	A 0.99 A
8	A 0.72 A	A 0.89 A	B 0.91 A	A 0.87 A	A 1.00 A
16	A 0.98 A	A 0.96 A	A 1.01 A	A 0.90 A	A 0.94 A
32	A 0.94 A	A 1.04 A	A 1.15 A	A 1.33 A	A 1.75 A
64	A 1.03 A	A 1.47 A	A 1.40 A	A 1.36 A	A 1.49 A

[a] Within the same row, values not followed by the same letter are significantly different ($P \leq 0.05$). Within the same column, values not preceded by the same letter are significantly different ($P \leq 0.05$).

($P \leq 0.05$) showed that this reduction was significantly lower than reductions achieved using contact times of 8 to 64 min in ozonated water. This value, however, was not significantly different than that of a control treatment of 4 min and ozone treatment of 2 min at 4 ppm, which showed reductions in *E. coli* O157:H7.

The reductions in population of *E. coli* O157:H7 on alfalfa seeds treated for 2 min in water initially containing 10 and 21 ppm of ozone were 0.90 $\log_{10}$ CFU/g (86.8%) and 0.78 $\log_{10}$ CFU/g (82.5%), respectively. These reductions were not significantly different from treatments containing the same initial concentrations of ozone for up to 64 min. A general observation for the 2- and 4-min treatments was that as the concentration of ozone increased, differences in percentage of cells inactivated were increasingly statistically insignificant.

Regardless of the concentration of ozone in ozonated water, the reductions in population of *E. coli* O157:H7 were not significantly different when treatment was applied for 8, 16, 32, or 64 min. This may have occurred as a result of a rapid decrease in ozone concentration on contact with the alfalfa seeds. A system to maintain a constant level of ozone in treatment water was therefore evaluated. The concentration of ozone in the water after treatment was not determined because of biosafety concerns and turbidity caused by release of insoluble seed components.

The percentage of germination of seeds treated in water initially containing 0 to 21 ppm of ozone is shown in Table 2. The percentage of germination of seeds treated in water (control) ranged from 86.2 to 95.7%, whereas germination percentage of seeds treated with ozone was between 74.7 and 95.7%. With the exception of seeds treated for 64 min in water containing 21 ppm of ozone, within ozone concentration, the treatment time did not have a significant effect on germination percentage. Low germination at the highest time-concentration combination may be caused by a higher aqueous ozone concentration remaining in contact with the seeds for a longer time, thereby allowing greater penetration into the cotyledons and hypocotyls. Although the exact mechanism of ozone penetration and its action on the seed interior are not known, the highly oxidative ozone

TABLE 2. *Percentage of germination of alfalfa seeds following treatment with water containing initial ozone concentrations of 0 to 21 ppm for up to 64 min*[a]

Contact time (min)	Percentage of germination				
	0 ppm	4 ppm	8 ppm	10 ppm	21 ppm
2	A 94.9 A	A 94.5 A	A 95.4 A	A 93.0 A	A 95.7 A
4	A 86.2 A	A 90.9 A	A 82.5 A	A 90.8 A	AB 91.7 A
8	A 91.4 A	A 95.6 A	A 93.1 A	A 92.1 A	AB 88.2 A
16	A 95.7 A	A 90.2 A	A 92.5 A	A 91.4 A	AB 80.0 A
32	A 91.1 A	A 91.3 A	A 94.2 A	A 90.0 A	AB 90.9 A
64	A 90.3 A	A 89.5 A	A 87.9 A	A 86.0 A	B 74.7 A

[a] Within the same row, values not followed by the same letter are significantly different ($P \leq 0.05$). Within the same column, values not preceded by the same letter are significantly different ($P \leq 0.05$).

TABLE 3. *Reduction in populations of* E. coli *O157:H7 on alfalfa seeds and percentage of germination of seeds treated by continuous sparging of ozone for up to 64 min*[a]

Contact time (min	Population reduction ($\log_{10}$ CFU/g)		Reductions ($\log_{10}$ CFU/g)[b]	Percentage of germination	
	Control	Sparging		Control	Sparging
2	B 0.71 B	A 1.11 A	0.40	A 95.8 A	A 91.3 A
4	A 1.23 A	A 1.27 A	0.04	A 95.0 A	A 85.3 B
8	A 1.27 A	A 1.33 A	0.06	A 94.4 A	A 93.2 A
16	A 1.43 A	A 1.38 A	−0.05	A 95.5 A	A 89.9 A
32	A 1.63 A	A 1.84 A	0.21	A 96.5 A	A 87.8 B
64	A 2.21 A	A 2.21 A	0.00	A 95.7 A	A 90.2 A

[a] Within population or percentage of germination, values in the same row not followed by the same letter are significantly different ($P \leq 0.05$). Within the same column, values not preceded by the same letter are significantly different ($P \leq 0.05$).

[b] Reductions compared with control ($[\log_{10} \text{CFU/g}]_{\text{sparging}} - [\log_{10} \text{CFU/g}]_{\text{control}}$).

may disrupt some essential germination mechanisms. Overall, the average percentage of germination of control seeds and seeds exposed to the four initial ozone concentrations (4, 8, 10, and 21 ppm) ranged from 86.9 to 92.0%. Since germination percentage of seeds treated with ozone was not substantially different than that of the control seeds, it is concluded that ozonated water did not have a detrimental effect on seed viability.

Continuous sparging treatment. Seeds were treated by continuous ozone sparging for the same contact times used in experiments in which ozone was not replenished during treatments. Table 3 lists the reductions in populations of *E. coli* O157:H7 on alfalfa seeds treated in water continuously sparged with ozone. Even at shorter contact times, reductions in population of *E. coli* O157:H7 on alfalfa seeds were higher than respective reductions on seeds treated in water that was not continuously sparged. This could be attributed to the continuous supply of ozone and simultaneous agitation caused by movement of ozone gas in the water. The lowest reduction in population was 1.12 $\log_{10}$ CFU/g (92.1%) on seeds treated for 2 min and the highest reduction was 2.21 $\log_{10}$ CFU/g (99.4%) on seeds treated for 64 min. The 2-min control treatment gave a reduction of 0.71 $\log_{10}$ CFU/g (79.5%), which was significantly less ($P \leq 0.05$) than reductions that resulted from all the other control and ozone treatments. Other water or ozone treatment times did not result in reductions in population of *E. coli* O157:H7 that differed significantly from each other.

There was no significant difference ($P > 0.05$) in percentage of germination of control or treated seeds, regardless of time of contact with water or ozonated water. The germination percentages of control and treated seeds were 94.4 to 96.5% and 85.3 to 93.2%, respectively.

Effect of ozone sparging followed by heat treatment. Since ozone sparging did not eliminate *E. coli* O157:H7 on alfalfa seeds, sparging followed by heat was tested as a multihurdle approach. Figure 1 shows the reduction in *E. coli* O157:H7 on alfalfa seeds subjected to continuous ozone sparging followed with heat treatment. Water (control) and ozone sparging treatment followed by heating at 60°C for 3 h resulted in the highest reductions in population of *E. coli* O157:H7. No colonies were formed on TSAN, indicating less than 40 CFU/g of seeds. A 48-h enrichment of 25 g of treated seeds in 100 ml of TSBN, however, revealed the presence of *E. coli* O157:H7 on treated seeds.

The average reductions in number of *E. coli* O157:H7 on ozone-treated seeds subsequently heated at 40 and 50°C were 0.19 to 2.53 $\log_{10}$ CFU/g (35.0 to 99.7%) and 0.46 to 3.58 $\log_{10}$ CFU/g (65.2 to 99.9%), respectively. These temperatures were not as lethal as treatment at 60°C.

Analysis of variance showed that there were no signif-

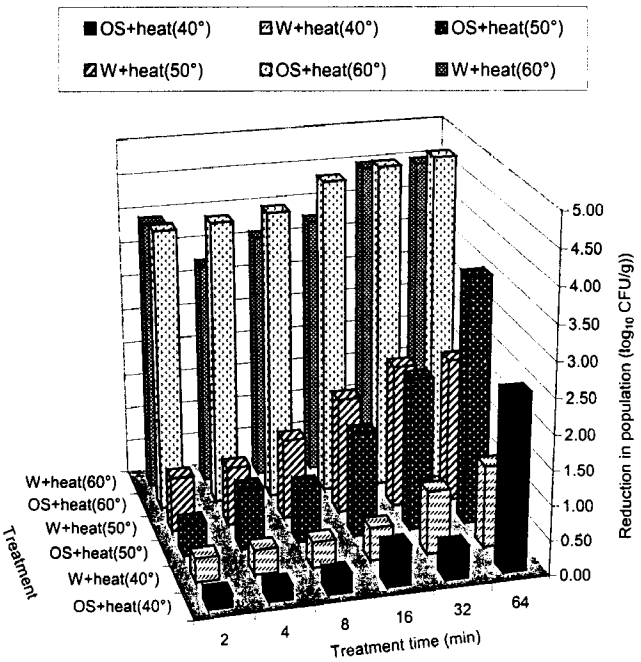

FIGURE 1. *Reduction in population of* E. coli *O157:H7 on alfalfa seeds subjected to continuous ozone sparging (OS) and deionized water (W) for up to 64 min followed by treatment at 40, 50, or 60°C for 3 h.*

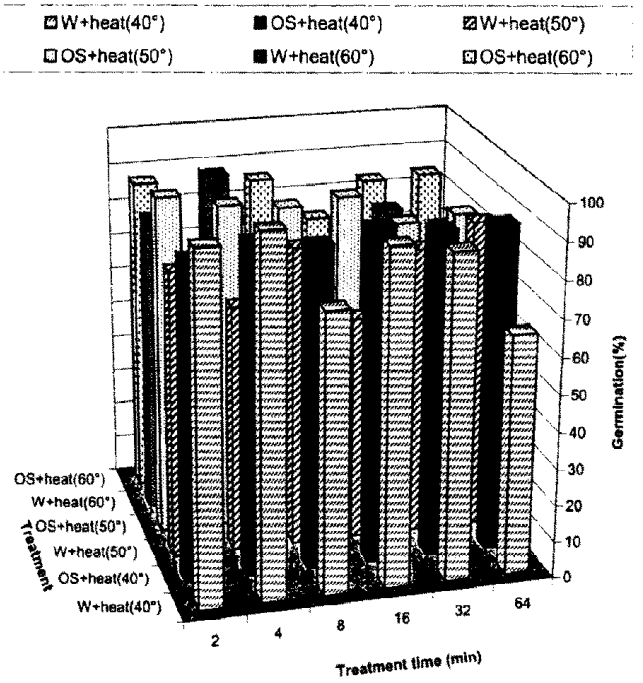

FIGURE 2. *Percentage of germination of alfalfa seeds after exposure to continuous ozone sparging (OS) and deionized water (W) for up to 64 min followed by treatment at 40, 50, or 60°C for 3 h.*

icant differences in percentage of germination of seeds sparged with ozone for the various contact times (Fig. 2). Treatment temperature did not significantly affect the germination percentage of ozone-treated and untreated seeds. This indicates that ozone, coupled with heat, did not kill the alfalfa seeds.

Overall, regardless of the mode of introduction of ozone to treatment solution, elimination of viable *E. coli* O157:H7 on alfalfa seeds was not achieved. Higher ozone concentrations and temperatures were more effective in reducing populations of *E. coli* O157:H7 on seeds; however, an increase in contact time of ozone with seeds did not necessarily give a greater reduction.

Susceptibility of microorganisms to ozone varies with the physiological state of cells, pH of the medium, temperature, humidity, and presence of other chemicals such as acids, surfactants, and sugars. Relatively low concentrations of ozone and short contact times are sufficient to inactivate cells in pure suspensions of bacteria, molds, yeasts, parasites, and viruses *(9)*. However, the presence of organic matter in foods rapidly reduces the ozone concentration of treatment solution, thereby diminishing antimicrobial effectiveness. Additionally, food may limit the accessibility of ozone to cells lodged in cracks, crevices, and damaged area on seeds, thereby affecting inactivation kinetics *(8)*.

Ozone is a strong biocidal agent, although this study reveals that its effectiveness in killing *E. coli* O157:H7 on alfalfa seeds is less than satisfactory. There is, however, a need to more thoroughly investigate ozone as a sanitizer for alfalfa seeds and sprouts since its potential lethality to foodborne pathogens was not fully evaluated in this study.

ACKNOWLEDGMENTS

Funding for this project was provided by the National Alliance for Food Safety, the U.S. Department of Agriculture, Agricultural Research Service (USDA Specific Cooperative Agreement no. 58-1935-0-022), and the Pennsylvania Agricultural Experiment Station. We also thank Hess Machine International (Ephrata, Pa.) for loaning the ozone generator used in this study.

REFERENCES

1. Bader, H., and J. Hoigne. 1981. Determination of ozone in water by the indigo method. Water Res. 15:449–456.
2. Beuchat, L. R., T. E. Ward, and C. A. Pettigrew. 2001. Comparison of chlorine and a prototype produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. J. Food Prot. 64:152–158.
3. Federal Register. 2001. Secondary direct food additives permitted in food for human consumption, final rule. Fed. Regist. 66:33829–33830.
4. Gordon, G., G. E. Pacey, W. J. Cooper, and R. G. Rice. 1988. Current state-of-the-art measurements of ozone in the gas phase and in solution. Ozone Sci. Eng. 10:353–366.
5. Graham, D. M. 1997. Use of ozone for food processing. Food Technol. 51:72–75.
6. Grunwell, J., J. Benga, H. Cohen, and G. Gordon. 1983. A detailed comparison of analytical methods for residual ozone measurement. Ozone Sci. Eng. 5:203–223.
7. Harmon, S. M., D. A. Kautter, and H. M. Solomon. 1987. *Bacillus cereus* contamination of seeds and vegetable sprouts grown in a home sprouting kit. J. Food Prot. 50:62–65.
8. Kim, J. G., A. E. Yousef, and G. W. Chism. 1999. Use of ozone to inactivate microorganisms on lettuce. J. Food Saf. 19:17–34.
9. Kim, J. G., A. E. Yousef, and S. Dave. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J. Food Prot. 62:1071–1078.
10. Lang, M. M., B. H. Ingham, and S. C. Ingham. 2000. Efficacy of novel organic acid and hypochlorite treatments for eliminating *Escherichia coli* O157:H7 from alfalfa seeds prior to sprouting. Int. J. Food Microbiol. 58:73–82.
11. National Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluations and recommendations on sprouted seeds. Int. J. Food Microbiol. 52:123–153.
12. Pryor, A., and R. G. Rice. 1999. Introduction to the use of ozone in food processing applications, p. 28–36. *In* 14th Ozone World Congress, Dearborn, Mich., 22–26 August.
13. Rice, R. G. 1999. Ozone in the United States of America—state-of-the-art. Ozone Sci. Eng. 21:99–118.
14. Rosas, C. J., and E. F. Escartin. 1999. Incidence and germicide sensitivity of *Salmonella typhi* and *Vibrio cholerae O1* in alfalfa sprouts. J. Food Saf. 19:137–146.
15. Rosas, C. J., and E. F. Escartin. 2000. Survival and growth of *Vibrio cholerae* O1, *Salmonella typhi* and *Escherichia coli* O157:H7 in alfalfa sprouts. J. Food Sci. 65:162–165.
16. Taormina, P. J., and L. R. Beuchat. 1999. Behavior of enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa sprouts during the sprouting process as influenced by treatments with various chemicals. J. Food Prot. 62:850–856.
17. Taormina, P. J., and L. R. Beuchat. 1999. Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. J. Food Prot. 62:318–324.
18. Taormina, P. J., L. R. Beuchat, and L. Slutsker. 1999. Infection associated with eating seed sprouts: an international concern. Emerg. Infect. Dis. 5:626–634.